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(54) Prefabricated MALDI layers suitable for storage

(57) Sample supports are provided with prefabricated matrix layers for matrix-assisted laser desorption and ionization of large analyte molecules (MALDI) for mass spectrometric analysis of the analyte substance. The sample supports, which may be flat plates or tiny magnetic beads, are made shippable and storable by using a matrix of at least two different components. A lacquer-like component protects the other substanc s from oxidation, hydrolysis and other types of decomposition. The other substances provide ionization of the analyte molecules during laser desorption. The lacquer-like component also absorbs the analyte molecules on the surface and assists in desorption during laser bombardment. Particularly favourable is a thin layer of nitrocellulose (more correctly: cellulose nitrate), in which the substances, such as cinnamic or similar acids, necessary for ionization of the analyte molecules are embedded. The water-insoluble lacquer layer adsorbs the large analyte molecules from the solution on its surface. The prefabricated layer can also contain other components required for modification of the analyte molecules, for example enzymes for specific digestion of proteins. Components may also be present for the selective binding of analyte molecules, such as antibodies for capturing specific proteins.

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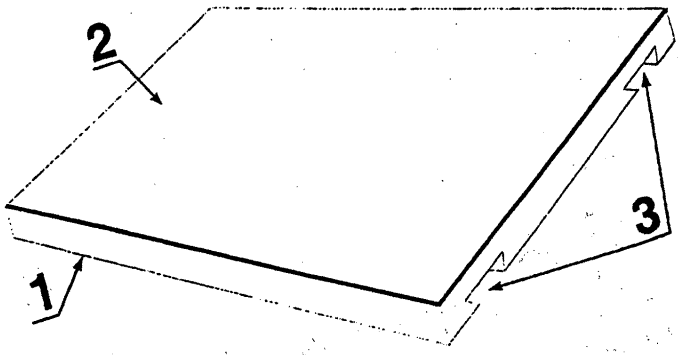

Figure 1

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Prefabricated MALDI Layers Suitable for Storage

The invention refers to sample supports with prefabricated matrix layers for matrix-assisted laser desorption and ionization of large analyte molecules (MALDI) for mass spectrometric analysis of the analyte substance, and methods for the production of the matrix layers on the sample supports.

In methods for mass spectrometric analyses of macromolecular analyte substances using ionization by laser-induced desorption, the sample support with an analyte substance at its surface is irradiated by a short light pulse from a laser that is focused on the sample surface. This light pulse generates ions from the analyte molecules which are then subjected to mass spectrometric analysis. In most cases, time-of-flight mass spectrometers are used for this analysis, but also ion storing mass spectrometers, i. e. RF quadrupole ion traps (often simply called "ion traps") or ion cyclotron resonance mass spectrometers ("ICR spectrometers"), have been applied to this task.

In the particular case of time-of-flight mass spectrometry, a high voltage between 6 and 30 kilovolts is applied to the sample support which – in the simplest case – faces a base electrode at ground potential at a distance between 10 and 20 millimeters. A laser pulse of typically about 4 nanoseconds duration performs the ionization. The ions are accelerated through the electrical field toward the perforated base electrode, whereby all ions receive the same kinetic energy. Beyond the base electrode is the drift region of the time-of-flight mass spectrometer. At the end of this flight path, the ions are detected at their arrival time and, since their kinetic energy is identical but their velocity is mass-dependent, their time of flight can be used to determine their masses.

When using ion storage mass spectrometers such as quadrupole ion traps or ICR spectrometers, the ions generated by the desorption process are transferred by ion-optical means into the storage cells of the mass spectrometers and are analyzed there by well-known mass spectrometric procedures.

For the ionization of large analyte molecules through the widely known matrix-assisted laser desorption (MALDI = matrix-assisted laser desorption and ionization), which has become widespread in recent years, the large molecules of the analyte substance on the sample support are embedded in a layer of tiny crystals of a low-molecular weight matrix substance. The laser light pulse vaporizes a small amount of matrix substance almost instantaneously. The vapor cloud first occupies virtually the same space as the solid substance, that is, it is under high pressure. Even the large analyte molecules are transferred into a gaseous state inside the initially tiny vapor cloud. During creation of the vapor cloud, a small portion of the molecules, that is both the matrix and the large analyte molecules, become ionized. After creation in a nanosecond time scale, the vapor cloud

immediately expands into the ambient vacuum in an adiabatic and isentropic process similar to an explosion, transforming pressure into speed. As long as contact still exists between the molecules during expansion of the vapor cloud, ionization of the large analyte molecules continues to take place due to ion-molecule reactions at the expense of the smaller matrix ions.

The vapor cloud expanding into the vacuum not only accelerates the molecules and ions of the matrix substance by its adiabatic expansion, but also the molecules and ions of the analyte substance due to viscous friction. Not all the molecules and ions end up with the same speed. The spread of initial velocities thus being produced during laser-induced ionization has a detrimental effect on and restricts the mass resolution of time-of-flight mass spectrometers. However there is a method of refocusing the ions and therefore improving the resolution.

For the other types of mass spectrometry mentioned, the spread of initial energies is also detrimental since it complicates the ion capture process in the storage cells.

For the MALDI process, the matrix substance must presently fulfill various tasks at the same time, whereby only a certain compromise can be achieved: it must catch and hold the analyte molecules (usually in its crystals during crystal growth) and retain them firmly on the sample support (through epitaxial growth of the crystals). It must absorb the light from the laser radiation effectively and thus collect enough energy for the instantaneous vaporization within a very short span of time. During vaporization, it must attain such a high plasma temperature that not too small a fraction of the molecules are ionized; on the other hand the matrix substance must not – such as through decomposition – lose its ionization enabling properties. It must then ionize the large analyte molecules through protonation in the subsequent ionization process.

These tasks are very difficult to fulfill evenly; the matrix substances are therefore of a very complex nature.

Previous preparation methods have required a fresh preparation, whereby analyte substance and matrix were applied at the same time in one solution onto the sample support and allowed to dry there. The purpose of this method was to enclose the analyte molecules in the microcrystals of the matrix substance which form when the applied droplet dries. Only a very few methods are known whereby the matrix substance is first applied to the sample support and then later the analyte substance, and these methods usually require at least partial dissolving of the previously applied matrix crystals (see for example GB 2 236 185).

Even with preparation of the matrix layer and later application of the analyte substance, the matrix layer must be relatively freshly applied. The reason for this is that the matrix substances usually are very sensitive to oxidation, hydrolysis and other altering processes

caused by components in the air, and therefore decompose easily. They can - especially when finely distributed on the surface of a carrier plate - no longer be used for the intended purpose of ionization after a few days at the latest. Even lengthier storage of matrix substances in bottles is critical and not recommended, once the bottle is opened and the content exposed to air.

These methods are therefore not suitable for prefabricating and then using large numbers of MALDI carrier plates. They are particularly not suitable for industrial prefabrication of marketable sample supports with MALDI layers.

Furthermore, methods have become known by which analyte molecules are modified in situ during application to the sample support, in order to increase the information content of the mass spectrometric analysis method. For example, proteins can be decomposed into segments by enzymatic digestion (e. g. tryptic digestion) in a characteristic manner so that by measuring the molecular weights of these fragments, immediate identification of the proteins applied is possible by using protein data banks. Pathological changes to a known protein can also be determined by such a digestion with subsequent measurement of the molecular weights of the fragments, whereby it is even possible to recognize the changed segment of the protein chain.

The enzymes necessary for this digestion are relatively stable. They could be added to a prefabricated matrix layer if they could be prepared to remain stable, and if the enzymes could be applied without direct contact to the usually acidic ionization substances on the matrix. However, since there is no storable matrix preparation, this method also requires a fresh mixture.

It is the main objective of the invention to find a well-functioning MALDI layer such that the sample support with MALDI layer can be prefabricated and stored for a longer period of time without loss of function. The prefabricated MALDI layers should allow application of the analyte molecules in a simple manner, for example in the form of automatically pipetted droplets. The analyte substances should be firmly adsorbed by the prepared layer in such a way that they are washable, in order to remove residue from buffer substances or salts. The layer should contain the matrix components used for the ionization of analyte substances protected from decomposition during transport or storage, and should ease the application of analyte molecules. It should particularly be possible to industrially manufacture and market prefabricated sample supports.

It is a further objective of this invention to allow preparations including of other reaction partners, for example enzymes for the characteristic digestion of proteins or other chemicals for specific modification of the analyte substances.

The invention should provide a MALDI method with a high yield of ions and high sensitivity in order to work with sample quantities of only a few femtomol. It should additionally allow MALDI processes to run automatically due to a uniform layer.

It is the basic idea of the invention to divide up the range of tasks that the matrix substance must fulfill between two or more substance components and, at the same time, to assign one substance component the additional task of protecting the other substance components. We have discovered that the analyte molecules and the ionizing matrix molecules need not already be in contact in the solid layer for a successful MALDI ionization process. The enclosure of the analyte molecules by the microcrystals of the matrix substance was formerly the goal of all preparation methods.

Sharing the tasks between two substances could, according to the basic idea of this invention, look something like this:

(1) a first matrix substance (a "binder") takes care of the adsorptive bonding of the analyte molecules to a preferably smooth surface, it takes care of the bonding to the base, it can take care of energy absorption from the laser light, it particularly takes care of the creation of the plasma cloud and additionally provides protection for the ionizing matrix components according to the basic idea of this invention;

(2) at least one other matrix substance (an "ionizer"), which is preferably molecularly dissolved in the first substance, takes care of the ionization of the analyte molecules in the plasma cloud; it can, to help out, also take care of energy absorption, if this is not performed by the first or by other matrix substances.

Protection of the ionizing matrix components can best be realized if the ionizer (or ionizers) can be completely enclosed within an airtight and watertight mass of the binder. It is therefore a further idea of the invention to use a lacquer-like processable binder which can dissolve the ionizer in a molecular solution into the lacquer mass. For example, a lacquer can be formed from fructose and water. Alternatively, sucrose can be used. The surface can be polymerised to form a protective layer, once the lacquer has been applied.

It is necessary for the MALDI process that the binder releases the ionizer's molecules during desorption. This can occur, for example, through complete vaporization. However, since lacquer-like layers are generally made up of polymer molecules, complete vaporization is not easily achievable. It is therefore a further idea of the invention that the binder can best accomplish the release of the ionizer's molecules if it decomposes into small molecules during laser light bombardment, i.e. the binder can be formed from an explosive lacquer such as a plastic explosive in a solvent. Especially advantageous here are polymer explosives which decompose exothermically into small molecules of water, carbon monoxide, carbon dioxide, nitrogen and hydrogen when heated by the laser beam. However, it need not be such a strong exothermic decomposition process as with explo-

sives, also weakly exothermic or even slightly endothermic decomposition processes can be used here, the energy supply of which originates from the laser light.

The binder must also be able to take on the task of adsorptive bonding of the analyte molecules, since the embedded molecules of the ionizer cannot perform this task. It is therefore a further idea of the invention to use highly adsorptive polymer structures here, such as are known for example from adsorption columns for cleaning high-molecular organic substances or blot membranes for blotting 2D electrophoretic separation.

An excellent combination of a highly adsorptive polymer structured substance with the desired explosive characteristic, suitable for producing a lacquer, is nitrocellulose (more properly: cellulose nitrate, DIN abbreviation: CN), the explosiveness of which may be further adjusted by the degree of nitration. For nitrogen contents between 10.5 and 12.5%, one speaks of cellulose dinitrate (collodium cotton); for those between 12.5 and 14.14%, of cellulose trinitrate (gun cotton). Both types deflagrate when heated; the strength of deflagration increases the higher the nitration. Cellulose nitrates consist of about 100 to 3,500 partially to fully nitrated glucose units.

The binder may take on the task of light absorption. This task can be fulfilled by a derivative of cellulose nitrate, in which absorptive molecule groups are embedded into the cellulose structure. For example, a cinnamic or benzoic acid or acid ester may be incorporated into the cellulose structure by reaction with the hydroxy groups of the cellulose nitrate. These groups may be the same as or different to the ionizer component. Through selection of the molecular groups, it is possible to adapt the wavelength of the laser used. However it is also possible to assign this task to a third matrix substance such as succinic acid, which absorbs in the infra red region. Cellulose nitrate is excellent for dyeing and can therefore be made non-transparent for any wavelength.

Very simply dissolved in acetone, the cellulose nitrate can be applied to the sample support as a lacquer layer by spraying, painting or printing. A smooth and uniform layer is produced which is consequently a prerequisite for the automatability of sample preparation and of MALDI ionization. A uniform layer thickness is also necessary for accuracy in the mass determination. Technically, the wide-spread nitrocellulose-based lacquers are produced from cellulose nitrate. Nitrocellulose-based lacquers usually have the less-nitrated cellulose dinitrate as their base material. Only the nitration of the cellulose allows dissolution of the resulting product in organic solvents.

The basic structure of the cellulose is particularly favorable for the superficial binding of the analyte molecules due to their specially strong adsorptiveness. Since nitrocellulose is not soluble in water, proteins, water-soluble polymers and other macromolecular analyte substances can be applied very easily to the lacquer layer from an aqueous solution. Nitrocellulose is often used for blot membranes; in contrast to other, mostly expensive blot

membranes, it has the disadvantage that analyte molecules adhere very tightly, for some analysis methods too tightly, to the surface. In the present case, this is an advantage. The aqueous solution of high-molecular weight analyte substances such as proteins contains not only the analyte molecules but frequently also stabilizing buffer salts and other components which are harmful to the ionization process. The firm adhesion of the analyte molecules and the water insolubility of nitrocellulose allows an easy and low-loss washing of the applied macromolecular analyte substance.

Explosives, with their exothermic decomposition, also simultaneously lead to a very constant cloud formation, which in turn results in favorable preconditions for high mass accuracy in time-of-flight mass spectrometry. Smaller energy differences in the laser light beam play a subordinate role. The explosive is applied so thinly here (down to only fractions of a micrometer), that no autogenous afterburning in adjacent areas occurs since the sample support has an intense cooling effect and quenches combustion. In contrast to normal MALDI, where a laser light focus diameter of 100 to 200 micrometers is preferred for stripping a thin layer of the matrix surface over a large area, focus diameters of 5 to 20 micrometers may be used for explosive MALDI. Here the entire layer within this diameter can be eroded down to the sample support.

Application of analyte molecules to the surface of the lacquer layer has the further advantage that the ions of the analyte molecules formed in this way have a much smaller spread of initial velocities after expansion of the cloud. The ions can therefore be captured much more efficiently in the storage cells of ion storage mass spectrometers, and they increase the accuracy of the mass determination in time-of-flight mass spectrometers.

To make the applied layer insoluble even to nonaqueous solvents, it is particularly advantageous to cross-link the usually threadlike molecules of the lacquer layer after application to the sample support, at least at the surface. This can be accomplished by the addition of a bridge forming agent, or also by ionizing radiation, for example with UV light. For the surface cross-linking of cellulose nitrate, diisocyanate has proven effective as a bridge building agent which joins remaining OH groups of adjacent molecule strands to one another. This cross-linking prevents solubility in organic solvents. Cross-linking does not prevent the decomposability of cellulose nitrate when subjected to laser radiation.

Suitable ionizers include alpha-cyano-4-hydroxy-cinnamic acid, 3,5-dimethoxy-4-hydroxy cinnamic acid, 2,5-dihydroxybenzoic acid, 3-hydroxy picolinic acid, 5-chlorosalycylic acid, and 2-(4-hydroxyphenylazo)-benzoic acid. A preferred ionizer is alpha-cyano-4-hydroxy-cinnamic acid (abbreviated as "alpha-cyano"). The skilled ad-

dressee will recognise that any suitable ionizer can be selected from known ionizers. The particular ionizer selected will depend on the required effect.

It has been shown in experiments that the concentration of the ionizer need not be high. With about 10% alpha-cyano in 90% nitrocellulose, an almost clear lacquer is produced which can be applied very thinly. It forms a good basis for the ionization of practically all types of proteins which can be easily applied to the surface of the water insoluble lacquer from an aqueous solution.

In the lacquer layer, several ionizing substances can be accomodated at the same time. They can be applied to the sample support as a single solution with several components, or also as a layered structure with several lacquer solutions on top of one another, each containing only one single ionizing component. The lacquer layers - either as a single component layer, a multicomponent layer or as a structure with several layers - can be sealed by a protective cover layer and made waterproof and airtight. The covered layer appropriately contains no ionizers. Cross-linking can be limited to the cover layer or even just to the surface of the cover layer.

For the MALDI process, it is necessary that the vapor cloud forms in an area of defined electrical potential. Since the lacquer layer is nonconductive, it has proven favorable to use a conductive sample support. In case a nonconducting sample support is used, surface metallization is recommended. This can be very thin, even almost transparent, in order to allow laser bombardment from the rear through the sample support.

To prevent charging of the lacquer layer, or in order to make the lacquer layer itself conductive, a conductor in disperse form can also be added to the lacquer. For example, carbon can be used for this in a very fine, dispersive form.

By making such layers conductive, it is possible to produce sample support plates which can be used directly as a blot membrane for blotting two-dimensionally through gel electrophoresis of separate proteins. The two-dimensional scanning by MALDI produces an increase in sensitivity compared to standard dyeing methods, which is several orders of magnitude greater and which furthermore offers dependable information in regard to the molecular weight.

If we disregard blotting, the analyte molecules are usually simply applied to the adsorptive layer by adding a tiny droplet of the analysis solution. The smooth lacquer surface thereby allows automatic pipetting of small droplets in which dissolved analyte molecules are found. The droplets retain a somewhat hemispherical shape on the lacquer layer from which the analyte molecules are removed in a relatively short time through diffusion and firm adsorption at the lacquer surface. Often the droplet does not even need to dry - after adsorption of the analyte molecules, the residue can simply be washed away. Also the substances from dried droplets can be washed off. This is particularly advanta-

geous since buffer substances and salts disturbing the MALDI process can be removed in this way.

The surface beneath a droplet of about 10 nanoliters solution is about 250 micrometers in diameter and can absorb, by order of magnitude, 10 picomol of analyte molecules, forming a monomolecular adsorption layer. If the concentration is much less than one picomol per microliter, the solution can be pipetted a number of times after each evaporation of the droplet - perhaps including several washing procedures. The area of the droplet can be ablated by several laser bombardments during the measurement procedure and produces a very good sum spectrum of the analyte substance by addition of several single spectra. Usually several single spectra can be recorded from a single sample spot. These single spectra, due to the uniformity of the analyte application, all have the same quality.

For highly diluted analyte solutions in which an applied droplet does not contain enough molecules for a mass spectrometric analysis, another type of sample support has proven effective. According to this invention the adsorptive matrix layer is applied to the surface of tiny magnetic beads which are available in diameters of 1 to 100 micrometers. It is particularly advantageous here to make the matrix layer completely insoluble by means of cross-linking. The beads are then added in a small number to the very dilute analyte solution. By lengthy contact with agitation, the analyte molecules can thus be practically bound to the surface of the beads in a quantitatively adsorptive process. If complete adsorption is not supposed to occur, the remaining analyte solution is not contaminated by released matrix substances. The beads can then be removed from the solution using special magnetic tools and applied to a flat sample support base. There they can be attached by magnetic forces, by superimposed very fine grids, or simply by adhesive bonding. After transfer to the vacuum, they are directly bombarded by the laser, providing an excellent MALDI. The beads may also be prefabricated so that they are shippable and storable.

The beads are especially effective for use if only minimal amounts of analyte substance are available, because they can absorb the analyte molecules almost completely even from very dilute solutions or the smallest volumes. Here the solution need not be pipetted or otherwise transported, thus keeping losses due to wall adsorption to a minimum. In this way, even analyte substances from single biological cells can be subjected to mass spectrometric analysis.

The MALDI methods previously employed essentially required irradiation of the sample support from the sample side, since only a very small amount of matrix at the surface was evaporated each time, therefore only a fraction of the layer thickness was eroded in each case. Even the use of magnetic beads requires irradiation from the sample side since the beads are nontransparent.

If, however, flat sample supports are used, a different method may be employed since the use of a thin layer of explosive lacquer leads to complete evaporation of a small layer area, so that a bare part of the sample support remains. It is therefore possible to irradiate the matrix layer from the rear of a sample support which is permeable to laser bombardment and evaporate it in this way. It is therefore a further idea of this invention to use transparent sample supports which, to nevertheless maintain stable electrical potentials, have been made conductive at the surface. The rear of the sample support is much more accessible for the laser beam in the ion source than the front, where the acceleration and focusing diaphragms, with their high voltages, prevent vertical bombardment or a bombardment with a short focal length, making very complicated designs necessary.

The wavelength of the laser, which was always very important for previous MALDI methods and which also determined the selection of matrix substances, is now only of secondary priority since the sole purpose of the laser beam is to ignite the explosive and generate a plasma of sufficiently high temperature.

Figure 1 shows an arrangement with a flat, metallic sample support 1 with the lacquer layer 2 of cellulose nitrate with embedded molecules of a substance such as 10% alpha-cyano-4-hydroxi-cinnamic acid, which is used for the ionization of the analyte molecules applied to the layer for the MALDI procedure. The sample support is 8 x 12 centimeters, the size introduced for microtiter plates, and is provided with dovetail guideways 3 with which the plate can be attached to a guide rail. The sample support is polished on its layer side in order to reflect the laser light beam and thus allow good absorption of the light.

A favorable embodiment is the flat metallic carrier plate shown in Figure 1 in the 8 x 12 centimeter size standardized for microtiter plates. This size fits in all current micropipette systems and therefore represents a favorable precondition for automatic sample application. The dovetail guideways allow attachment to movable sample tables, even in the vacuum of the mass spectrometer where the plate is simply held by these guides. Guides which have been surface treated with tetrafluoroethene ("Teflon™") have proven effective for use in a vacuum.

According to the invention, this plate is coated with the matrix layer on the sample side. Here it is assumed that the lacquer consists of cellulose nitrate dissolved in acetone with a nitration degree of about 12% and a chain length ("viscosity") of about 100 to 200 glucose groups and a small share of alpha-cyano-4-hydroxi-cinnamic acid, which has also been dissolved in acetone. The layer should be very thin, measuring only a few micrometers. Two methods have proven specially effective for application: the well-known electrospray ionization and the application of a droplet to the quickly rotating carrier plate. Application first occurs in an atmosphere with saturated acetone vapor, into which heated clean air is blown suddenly. Drying then takes place in a few seconds, creating a

very uniform layer. Cellulose nitrate without alpha-cyano-4-hydroxi-cinnamic acid or other ionizing substances has no ionizing effect during the MALDI process.

The addition of about 10% of alpha-cyano-4-hydroxi-cinnamic acid has been able to ionize all peptides and proteins used as analyte molecules in our tests up to now. The skilled addressee may however select any suitable ionizer, having regard to the reaction conditions. One can use slightly different types of sample support types. However, it is also possible to use different ionizers in the same matrix mixture. Should the different ionizers not be stable in the same solution, a layered structure can be selected which contains layers with different ionizers. For the user, it is advantageous if a single carrier plate type can be used for all analytical tasks.

These carrier plates are also suitable for the quick analysis of very large numbers of samples (so-called "massive-parallel processing"). With one millimeter of spacing, 9,600 sample spots can be applied and analyzed on one carrier plate. With a spacing of one half millimeter, 38,400 samples can be applied. If one assumes 2 seconds of analysis time per sample spot, these 38,400 samples can be analyzed in about 20 hours. This goal has been set for medical series analyses. Automatic equipment for rapid application to carriers with such large sample numbers is currently being developed.

These carrier plates require a concentration of analyte substance of about 1 picomol per microliter. Converted into weight concentrations, this corresponds approximately to a proportion by weight of a millionth of solvent (1 ppmw). That is a low concentration, lower than normally results from chromatographic or electrophoretic separation methods.

However, should the concentration used be much lower, a completely different type of sample support can be used which consists of magnetized beads of about 10 micrometers in diameter. These are also coated with a lacquer-like layer of material and can be stored like the carrier plates for longer periods of time. A small number of beads is then added to the very dilute solution for a longer time and heavy agitation. The adsorptive surface of the beads then picks up the analyte substance from the solution and captures it. The beads can then be removed from the solution with special magnetic tools, washed and applied to a flat base. Here they are attached and fed to the MALDI process.

The beads can be coated very easily with a thin matrix layer by allowing them to fall individually through a very fine (electrically generated) lacquer spray jet. Having passed the lacquer spray jet, the beads are dry after passing along a path of about 20 centimeters of dry air and can then simply be gathered in a glass. Setting up a whirled air stream in the glass prevents the freshly coated beads from sticking together. - Pneumatic spraying of the beads together with a very dilute lacquer solution also leads to a very thin lacquer layer on the beads.

It has been a frequently occurring, continuous analytical task to remove the analyte substance specifically from a solution mixture in which not only the analyte substance of interest is found. For example, one would like to remove a protein of interest from a protein mixture and analyze it for malformations. This task, as is known, can be performed by antibodies. These antibodies can be applied relatively easily to the lacquer-like matrix layer and chemically bound there without destroying the bonding properties of the antibodies. Such prefabricated sample supports are then quite specifically suited for the analyte of certain proteins. Especially those sample supports taking the form of beads can be used in this way. It will be possible, for example, to remove a very specific protein from one single cell and analyze it.

However, the specific modification of analyte molecules can also be achieved in this way. Thus it is favorable for the identification of proteins, for example, to subject the protein to an enzymatic digestion which undoes the chain of amino acids right between or right after precisely defined amino acid pairs. Thus, for example, the enzyme trypsin makes a separation after the amino acid pair of lysine and argenine. The molecular weights of the sections from the tryptic digestion very quickly permit identification via a protein data bank. This digestion can now be performed in situ on the sample support. To do this, it is necessary to have trypsin (or a different enzyme) adsorbed or otherwise solidly bound to the sample support. Since the enzymes are relatively stable, they can be stored for a longer period of time on a sample support. However, they must be kept apart from the normally acidic ionizers, which is ensured however by the lacquer-like structure of the layer. For this type of preparation with mutable biochemicals, either flat carrier plates or magnetizable beads are suitable.

CLAIMS

1. A sample support for supporting an analyte in the MALDI process, comprising a matrix layer having at least one ionizing component for ionizing the analyte during laser desorption, and a protecting component for protecting the ionizing component, wherein the protecting component reduces the contact between the ionizing component and the atmosphere, thereby at least partially preventing degradation of the ionizing component.

2. A sample support as claimed in Claim 1, wherein the ionizing component is dissolved in the protecting component.

3. A sample support as claimed in Claim 1 or Claim 2, wherein the protecting component is in the form of a lacquer.

4. A sample support as claimed in any one of the preceding claims, wherein the protecting component is a substance which undergoes molecular decomposition on application of laser light.

5. A sample support as claimed in Claim 4, wherein the protecting component decomposes into molecules of water, carbon monoxide, carbon dioxide, nitrogen, hydrogen, or a combination thereof, on application of laser light.

6. A sample support as claimed in any one of the preceding claims, wherein the protecting component has a polymer structure.

7. A sample support as claimed in any one of the preceding claims, wherein the protecting component has a structure to which, in use, the analyte molecules are bound.

8. A sample support as claimed in any one of the preceding claims, wherein the protecting component is cellulose nitrate.

9. A sample support as claimed in Claim 8, wherein the degree of nitration of the cellulose nitrate is from 10.5 to 14.14 percent.

10. A sample support as claimed in Claim 9, wherein the degree of nitration of the cellulose nitrate is from 11.5 to 13 percent.

11. A sample support as claimed in any one of the preceding claims, wherein the protecting component is at least partially cross-linked.

12. A sample support as claimed in Claim 11, wherein the cross-linking is a result of irradiation.

13. A sample support as claimed in Claim 11, wherein the cross-links are provided by a bridge-building agent.

14. A sample support as claimed in Claim 13, wherein the bridge-building agent is di-isocyanate.

15. A sample support as claimed in any one of the preceding claims, wherein the protecting component is cellulose nitrate reacted with a cinnamic or a benzoic acid.

16. A sample support as claimed in any one of the preceding claims, additionally comprising a further component which absorbs the laser light during the desorption process.

17. A sample support as claimed in any one of the preceding claims, wherein the ionizing component is alpha-cyano-4-hydroxy-cinnamic acid, 3,5-dimethoxy-4-hydroxy cinnamic acid, 2,5-dihydroxybenzoic acid, 3-hydroxy picolinic acid, 5-chlorosalycylic acid, or 2-(4-hydroxyphenylazo)-benzoic acid.

18. A sample support as claimed in any one of the preceding claims, wherein the ionizing component is alpha-cyano-4-hydroxy-cinnamic acid.

19. A sample support as claimed in any one of the preceding claims, wherein the said matrix layer comprises approximately 90% w/w of the protecting component and approximately 10% w/w of the ionizing component.

20. A sample support as claimed in any one of the preceding claims, wherein the said matrix layer has one ionizing component only.

21. A sample support as claimed in any one of Claims 1 to 19, wherein the said matrix layer includes a plurality of different ionizing components, substantially homogeneously distributed within the protecting component.

22. A sample support as claimed in any one of Claims 1 to 19, wherein the said matrix layer includes a plurality of layers of the protecting component, each layer having a different ionizing component distributed therein.

23. A sample support as claimed in any one of the preceding claims, wherein the said matrix layer has a sealing cover layer, which comprises the protecting component and which contains no ionizing component or a negligible amount only of the ionizing component.

24. A sample support as claimed in any one of the preceding claims, wherein the said matrix later additionally comprises an electrically conducted component which renders the said matrix layer electrically conductive.

25. A sample support as claimed in Claim 24, wherein the said conductive component is carbon powder.

26. A sample support as claimed in any one of the preceding claims, wherein the said matrix layer is carried on a carrier plate.

27. A sample support as claimed in any one of the Claims 1 to 25, wherein the said matrix layer is carried on a plurality of magnetic beads.

28. A sample support as claimed in any one of the preceding claims, which has a reagent bound to the said matrix layer which reagent, in use, chemically modifies the analyte.

29. A sample support as claimed in Claim 28, in which the reagent is an enzyme.

30. A sample support as claimed in any one of the preceding claims which has an agent bound to the said matrix layer, which agent, in use, retains a specific analyte molecule.

31. A sample support as claimed in Claim 30, in which the agent is an antibody.

32. A sample support as claimed in any one of the preceding claims, wherein, in use, the analyte is bound to the protecting component.

33. A sample support as claimed in any one of the preceding claims, wherein the protecting component at least partially surrounds the ionizing component.

34. A method for the preparation of a sample support as claimed in Claim 26, wherein a solution of the protecting component is applied by spraying, printing, or painting onto the carrier plate, or by dripping onto a rotating carrier plate.

35. A method for the preparation of a sample support as claimed in Claim 27, wherein the beads fall through a fine jet of a solution of the protecting component, and are then dried in an air stream.

36. A method for the mass spectrometric analysis of a macromolecular analyte, using ionization by laser-induced desorption, wherein the said analyte is supported on a sample support as claimed in any one of Claims 1 to Claim 33.

37. Sample support for the MALDI process of analyte ionization with a prefabricated surface layer consisting of a matrix with at least two components, wherein those matrix components which are used for the ionization of the analyte molecules during the desorption process are densily surrounded and protected from changes during storage by one of the matrix components.

38. The use of magnetic beads as a carrier body for supporting a matrix layer in the MALDI process.

39. The use of a carrier body for supporting a matrix layer in the MALDI process which carrier body is transparent to laser light.

The Patent Office

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Applicati n No: GB 9709152.4 **Examiner:** Martyn Dixon
Claims searched: 1-37 **Date of search:** 18 June 1997

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Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): H1D (DHBB,DMG,DMH); G1B (BCF,BCH)

Int Cl (Ed.6): H01J (27/02,27/24,27/26,49/02,49/04,49/10,49/14,49/16); G01N (1/00,1/28,1/36)

Other: online: WPI,JAPIO,INSPEC

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A,P	GB 2299445 A	(Bruker-Franzen) see e.g. page 3, lines 11 et seq	1,37
A	GB 2287315 A	(Bruker-Franzen) see e.g. page 12, lines 14-21	1,37
A,P	WO 96/33797 A	(MIT) see page 21, lines 17-30	1,37
X	WO 95/15001 A	(Waters) see especially example 2 and page 9, line 14	1-8,17,18, 20,26,33, 36,37

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
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